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Observations on Tracheary Elements in *Salpichlaena* J. Sm. (Blechnaceae, Pteridophyta)

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Abstract.—The morphology of the tracheary elements of the climbing fern *Salpichlaena* (Blechnaceae) were analyzed by means of LM and SEM. Two preparative techniques were employed: maceration and sectioning. Under SEM macerated tracheary elements from roots, rhizomes and leaf rachises showed large scalariform perforations lacking pit membranes, thus constituting apparent perforation plates. The perforations showed terminal as well as lateral positions. "Multiple end-wall" and "intermittent" perforation plates were also observed. In sectioned material tracheary cells exhibited mostly intact pit membranes conforming tracheids with scalariform and circular to oval wall pitting. In roots, true perforations seemed to be present in some tracheary cells. A different degree of pit membrane perforations were registered in both macerated and sectioned material, thus suggesting the existence of "incipient vessel elements". According to our observations macerations produce alterations in the xylem tissue, which can lead to misinterpretations.

Key Words.—*Salpichlaena*, xylem, tracheids, pit membrane, incipient vessel elements

The morphology of the tracheary elements in ferns has been described by various researchers using light microscopy (e.g. Duerden, 1940; Bierhorst, 1958, 1960; White, 1960; Wilder, 1970). These authors found that fern xylem consisted mainly of tracheids with scalariform pitting and, in less proportion, vessel elements. The majority of these observations were performed on sectioned material. The presence of vessel elements was documented in genera such as *Pteridium*, *Selaginella*, *Equisetum* and *Marsilea* and consisted of cells with scalariform to simple perforation plates (Duerden, 1940; Bierhorst, 1958; White, 1960).

In the last decades, Carlquist and Schneider (1997a, b, 1998a, b, 1999, 2000a, b, c, 2001), Carlquist *et al.* (1999, 2000) and Schneider and Carlquist (1997, 1998, 1999a, b) documented the presence of vessel elements in all groups of ferns that they studied. Their interpretations were based on scanning electron microscope (SEM) observations from dissociated material. According to these authors, fern vessel elements showed features such as terminal and lateral scalariform perforation plates, "multiple end-wall" and "intermittent" perforation plates and circular, oval or scalariform pitting on the lateral walls. They also reported the presence of "porose pit membranes" in some ferns,

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considering them indicative of the transition between lateral wall pitting and perforations (Schneider and Carlquist, 1999; Carlquist and Schneider, 2000a, b, c; Carlquist *et al.*, 2000). As a conclusion of their research they stated that, at present, fern xylem seems to consist mostly or wholly of vessel elements (Carlquist and Schneider, 2001).

Nevertheless pit membranes are sometimes thought to be easily broken during maceration. Thus, various authors studied the morphology of the pit membranes in angiosperms under SEM from sections of both living and herbarium specimens (Carlquist, 1992; Dute *et al.*, 1992, 1996; Schneider and Carlquist, 1995; Feild *et al.*, 2000; Sano, 2004). Pit membranes appeared always intact and, in some instances, small holes were present.

The subject of this study, *Salpichlaena* J. Sm. (Blechnaceae), is a climbing fern that grows in tropical wet and rain forests of Central and South America. The rhizomes are long- creeping and scaly, and the fronds can reach up to ca. 15 m in length (Smith, 1995; Moran, 1995; Giudice *et al.*, In press.). Veres (1990) studied the xylem characteristics and hydraulic conductance of *Salpichlaena volubilis* (Kaulf.) J. Sm. and described the tracheary elements as exceptionally long tracheids (some > 4 cm) with both very large diameter (some > 200 μm) and large pit aperture areas between them.

Because the morphology of the tracheary elements in ferns stresses the need of a re-examination using different techniques, especially to observe the morphology of the pit membrane, the aim of the present work is to study the structure of *Salpichlaena* tracheary elements by employing different techniques for SEM observations.

Materials and Methods

The study was based on herbarium material of *Salpichlaena volubilis* (Kaulf.) J. Sm and *S. hookeriana* (Kuntze) Alston. Living tissue of this species was not available to study. Portions of roots, rhizomes and leaf rachises were obtained for light microscopy (LM) and scanning electron microscopy (SEM) analysis.

Part of the herbarium material was macerated according to Jeffrey's technique (Jeffrey, 1917). Samples were placed in solution for 12 hours at room temperature and then washed with distilled water. For SEM observations material was attached to aluminum stubs using double stick tape, air dried and sputter-coated with gold-palladium.

Split transverse and longitudinal sections were also obtained from herbarium specimens. Sections were placed in 80% ethanol and then in 90% ethanol, followed by absolute ethanol and finally were allowed to air dry (Dute *et al.*, 1992). Samples were then mounted on stubs as described above and sputter-coated with gold-palladium. Some herbarium specimens were split and left untreated. Observations were made in a JEOL, JSM-35 CF scanning electron microscope operated at 10 kv.

Tracheary element lengths were calculated from macerated material by employing a Nikon Photolab 2 light microscope. As root tracheary elements were scarce and appeared sectioned, only rhizome and leaf rachis elements could be measured.

Specimens Studied.—*Salpichlaena volubilis* (Kaulf.) J. Sm: LESSER ANTILLES: Guadalupe Island, *Proctor 20130* (GH); Idem, *Barrier 2885* (P); Dominica Island, *W.H. Hodge & B.T. Hodge 3482* (GH). Costa Rica: Pcia. Cartago, Cervantes, *Scamman 7179* (GH); Pcia. San José, San Isidro de El general, *Scamman 6037* (GH). PANAMA: Canal Zone, *Nee & Smith 11112* (NY), Co. Campana, *Madison 768* (GH). BRITISH GUIANA: Roraima, *Prance et al. 21612* (NY). ECUADOR: Pcia. Pastaza, *Holm-Nielsen & Jeppesen 445* (GH); Pcia. Napo, *Moran et al. 6034* (NY), Pcia. Imbabura, Rio Cachaco, *Sperling & Bleineiss 5068* (GH), Pcia. Esmeraldas, Río Cayapa, *Kvist & Asanza 40763* (GH). PERU: Depto. Loreto, Pcia. Maynas, *Tryon & Tryon 5178* (GH, F). BRASIL: Edo. Amazonas, Rio Amazonas, *Conant 930 et al.* (GH, NY), Edo. Mato Grosso, *Windisch & Oliveira 6485* (NY); Edo. Sao Paulo, Río Grande, *Rosenstock 192* (SI), Idem, Alto da Serra, *A. Tryon & R. Tryon 6592* (GH).

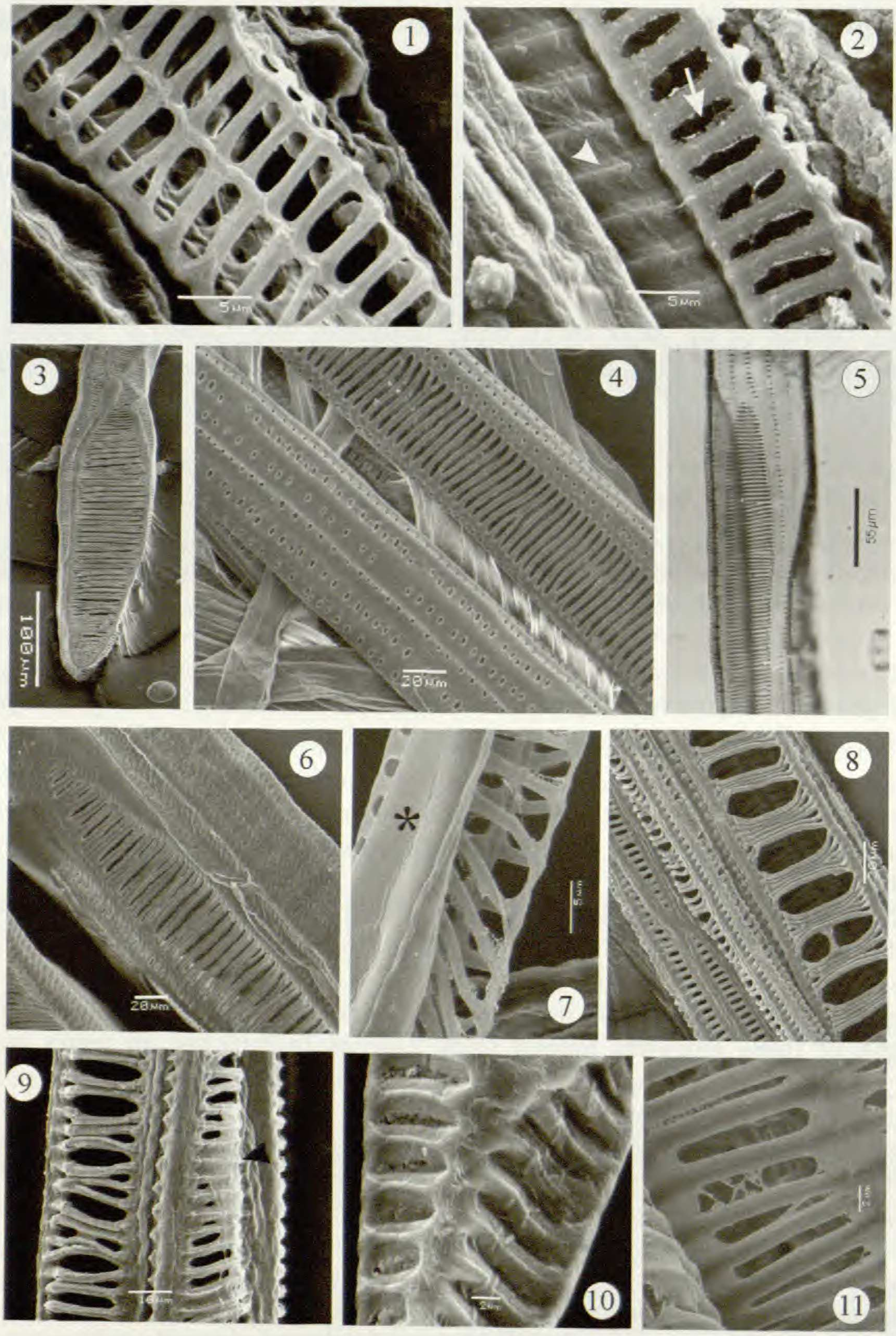
Salpichlaena hookeriana (Kuntze) Alston: PERU: Depto. Loreto, Pcia. Maynas, *Tuomisto et al. 10083* (NY).

Results

Under SEM macerated material from roots showed tracheary elements with various facets, mostly with monomorphic perforations (Fig. 1). In a few instances facets with intact pit membranes conforming to scalariform pits were observed, whereas other facets showed holes with remnants of torn pit membranes (Fig. 2).

Macerations from rhizomes and leaf rachises contained tracheary elements with a distinctive scalariform pattern of secondary wall deposition on their tips (Fig. 3), which differed from the circular to oval pattern of the lateral walls (Fig. 4). In both instances pit membranes were mostly lacking (Figs. 3 & 4). The length of these elements ranged from 2.03 cm to 5.65 cm. In many cases tracheary elements showed the so called "multiple end-wall perforation plates" and "lateral wall perforation plates" (according to Carlquist & Schneider, 2001; Figs. 5 & 6). The former characteristic was better observed with LM (Fig. 5). Tracheary cells with imperforated facets (Figs. 7) as well as with "intermittent perforation plates" (according to Carlquist and Schneider, 2001) were also observed (Figs. 8). As occurred in roots, remnants of pit membranes, from intact to porose and threadlike membranes, were present on some facets of the tracheary elements (Figs. 9–11).

Longitudinal sections from roots showed tracheary elements with circular, oval and scalariform pits with intact pit membranes, as well as cells with porose to thread like pit membrane remnants (Figs. 12–17). In some instances pit membranes seemed to be torn away during sectioning (Fig. 12, narrower cells at left). By contrast, other cells showed facets with apparently perforated



FIGS. 1–11. Tracheary elements from macerations of *Salpichlaena*. Figs. 1 & 2: Root tracheary elements. Figs. 3, 5, 7–9. Rhizome tracheary elements. Figs. 4, 6, 10–11: Leaf rachis tracheary elements. Fig. 1. Portion of one element showing large "monomorphic perforations". Fig. 2. Facets that illustrate intact pit membrane (arrowhead) and remnants of torn pit membrane (arrow). Fig. 3. Tip of a tracheary element with an apparent scalariform perforation plate. Fig. 4.

portions (Figs. 13–14, arrows). In Fig. 14 the smooth edges of the pit membrane remnants seem to indicate that they were not torn during sectioning. Porose and "weblike" to "threadlike" pit membrane remnants were observed in few occasions (Fig. 15–17).

Longitudinal sections from rhizomes showed tracheary elements mostly with scalariform pitting with intact pit membranes (Figs. 18–21). In Figs. 18 and 19, the so-called "terminal" and "lateral perforation plates" are shown. When pit membranes were absent, the presence of irregular shaped remnants on the sides of the pits indicated that they were torn away during sectioning (Figs. 20 & 21). Transverse and longitudinal sections of leaf rachises also exhibit the scalariform pitting of the tracheary cells (Figs 22 & 23).

Discussion

SEM analysis of *Salpichlaena* tracheary elements showed many morphological differences depending upon the technique employed for sample preparations. Macerated tracheary elements usually showed long cells with scalariform perforations on their terminal walls, thus conforming to "terminal scalariform perforation plates". As a consequence, these cells would be described as vessel elements. Perforations were also present on the lateral walls, corroborating the presence of "lateral wall perforation plates". Other features such as "multiple end wall perforation plates" and "intermittent perforation plates" were observed in dissociated samples. However, in *Salpichlaena* macerated specimens pit membranes were frequently lacking from the lateral wall pits. In this manner, if membranes are absent, we should describe those pits also as perforations. All the characters observed in macerated material are in agreement with the observations on tracheary elements in other ferns by Carlquist and Schneider (2000a, 2000b, 2001) and Carlquist *et al.* (2000).

The characters of *Salpichlaena* tracheary elements differed when material was sectioned. In this case, tracheary cells showed mostly intact pit membranes in terminal as well as lateral wall pitting. This character was frequently observed in xylem from rhizomes and leaf rachises. As a consequence these cells are described in the present work as tracheids with both scalariform and circular to oval wall pitting. Carlquist and Schneider

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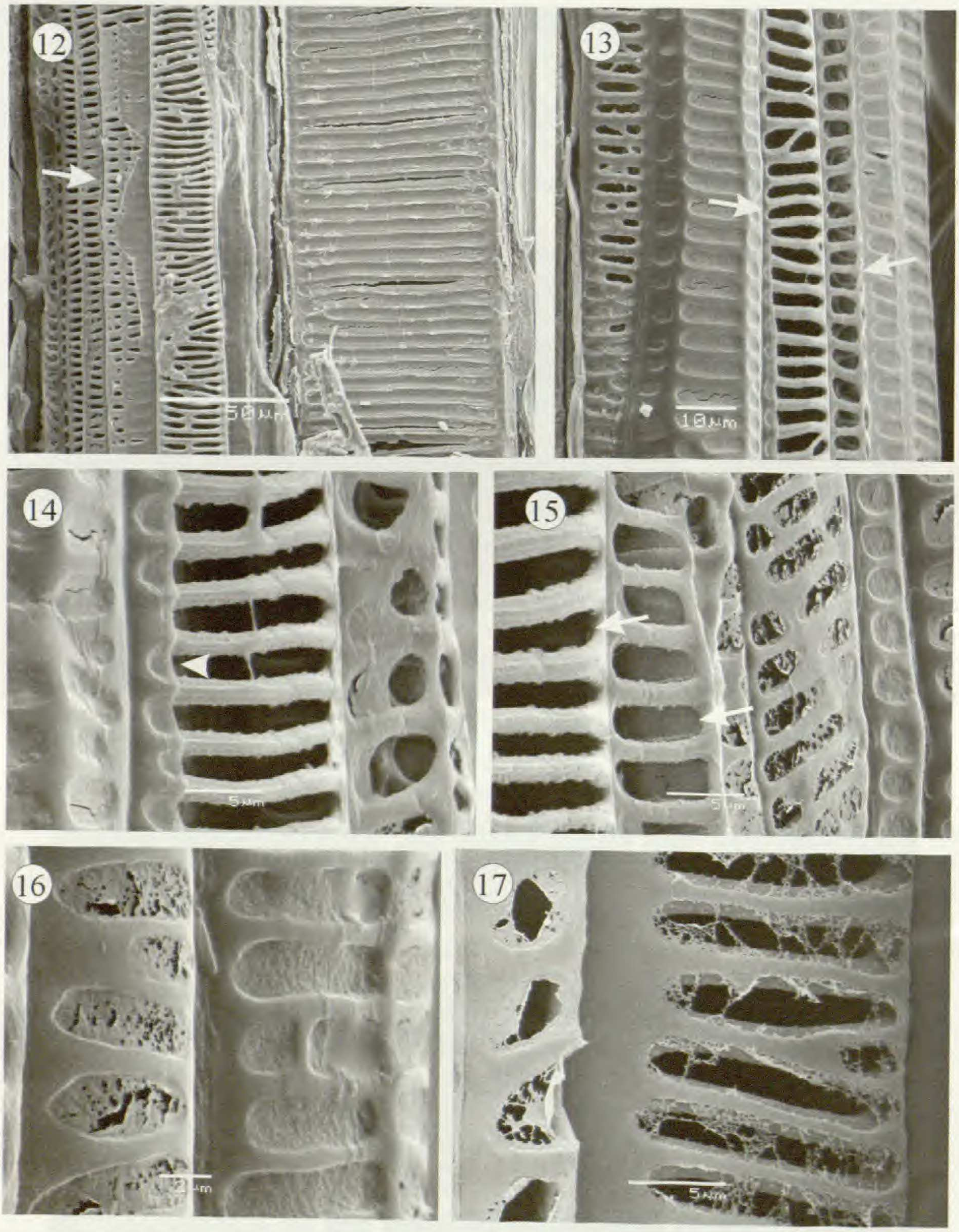
Facets of tracheary elements showing circular pitting (left) and scalariform pitting (right). Fig. 5. LM micrograph of element portion with "multiple end-wall perforation plates". Fig. 6. "Lateral-wall perforation plate" in one facet of a tracheary element. Fig. 7. Two facets of one cell; at left without pitting (asterisk) and at right with large perforations and remnants of pit membranes. Fig 8. Lateral wall of a tracheary element showing pit dimorphism ("intermittent perforation plate"). Fig. 9. Portions of intact pit membranes in scalariform pits (arrowhead) near the cell tip. Fig. 10. Porose pit membranes in the tip of a tracheary element. Fig. 11. Wall facet with perforations and remnants of thread-like pit membrane.



FIGS. 12–17. Tracheary elements from sections of roots of *Salpichlaena*. Fig. 12. Longitudinal section showing tracheary elements with intact pit membranes (right) and with remnants of pit membranes (left). In the latter, the primary wall material was torn away during sectioning (arrow). Fig 13. Portions of tracheary cells showing some facets with intact pit membranes and others with possible perforations (arrows). Holes at left seem to be artifacts. Fig. 14. Detail of possible perforations showing remnants of pit membranes with smooth edges (arrowhead). Fig. 15. Facets of tracheary elements that illustrate possible perforations (arrows). Web-like to threadlike pit

(2000) described the occurrence of tracheids in rhizomes and probably in roots of *Ceratopteris*, coexisting with vessel elements.

Differering from those of the rhizomes and leaf rachises, *Salpichlaena* root tracheary elements showed portions of walls which seemed to be perforations. In these observations, no remnants of torn pit membranes, which could indicate failures during preparation as well as during SEM observations, were observed.

Macerated samples of *Salpichlaena* root, rhizome, and leaf rachis tracheary cells showed different degrees of pit membrane perforation as found by Carlquist and Schneider (2000b) and Carlquist *et al.* (2000) in other ferns. In both studies some of the perforations seemed to be artifacts of the preparative techniques and perhaps the action of the SEM electron beam, judging from the fact that some portions of the remnants showed large holes or tears.

In this manner, our findings in sectioned samples from *Salpichlaena* are in agreement with the research of Veres (1990). The author studied the xylem of this fern in relation to hydraulic conductance and established that the xylem of *Salpichlaena* consisted of very long tracheids (some of them up to 4 cm) with a large pit aperture area between them. The author described the tracheary elements under LM and SEM employing both techniques of maceration and sectioning. Unfortunately only LM images, mostly of transverse sections, are given in his work. Additionally, the characteristics observed in *Salpichlaena* tracheary cells from herbarium specimens are similar to our observations from fresh material of *Asplenium* sp. from North-West Argentina (*results ined.*).

Our results suggest *Salpichlaena* tracheary elements consist mainly of tracheids with scalariform and circular to oval pitting. Other elements, in which a different degree of perforations are seen in some portions, might indicate the occurrence of incipient perforation plates. Our results also suggest that Jeffrey's fluid caused the rupture of the pit membranes in *Salpichlaena* tracheary elements in the majority of the cases. If vessel elements are present in this fern, they seem to be restricted only to roots. However, a more detailed study employing transmission electron microscope (TEM) techniques, and perhaps using fresh material, is necessary to establish the nature of such perforations.

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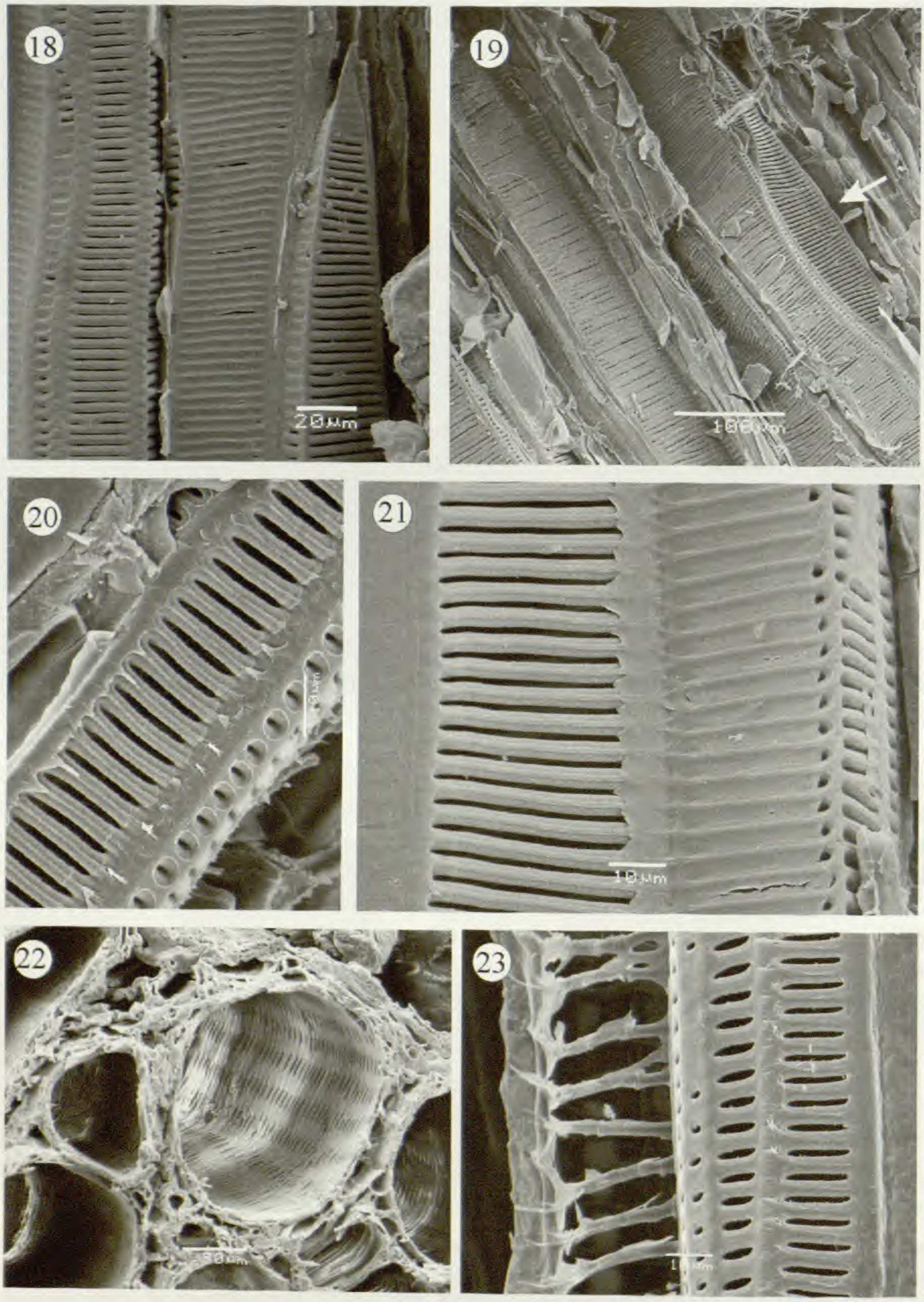
membrane remnants are seen in facets at center whereas circular pits are seen at right. Fig. 16. Portions of tracheary cells showing porose pit membranes (left), some of them with holes that are likely to be tears. At right scalariform pits with intact pit membranes. Fig. 17. Facets of tracheary elements with pit membrane remnants. At left pit membranes are torn. At right the remnants of pit membranes are web-like to thread-like.



FIGS. 18–23. Tracheary elements from sections of rhizome and leaf rachis of *Salpichlaena*. Figs. 18–21. Rhizome tracheary elements. Figs. 22–23. Leaf rachis tracheary elements. Fig. 18. Tracheary cells showing facets with scalariform pitting. At right a tip of a tracheary element is seen. Pit membranes were torn away during sectioning. Fig. 19. Scalariform pitting on lateral walls. At right

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a facet shows an apparent "lateral wall perforation plate" (arrow). Fig. 20. Facets of a tracheary element with scalariform and circular pitting. Fig. 21. Detail of intact and torn pit membranes. Fig. 22. Transverse section of tracheary cells showing scalariform pitting. Fig. 23. Protoxylem elements (helical rings) to metaxylem cells (scalariform pitting) transition to exhibit those primary walls were torn away.

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